

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C. 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 April 2000 (19.04.00)	
International application No. PCT/DK99/00458	Applicant's or agent's file reference P9757PC00
International filing date (day/month/year) 01 September 1999 (01.09.99)	Priority date (day/month/year) 02 September 1998 (02.09.98)
Applicant GREGERSEN, Soeren	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

09 March 2000 (09.03.00)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer Nestor Santesso</p> <p>Telephone No.: (41-22) 338.83.38</p>
--	---

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P9757PC00	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/DK 99/ 00458	International filing date (day/month/year) 01/09/1999	(Earliest) Priority Date (day/month/year) 02/09/1998
Applicant LANGERHANS APS et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

ST/DK 99/00458

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12M1/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	✓ EP 0 195 088 A (SUMITOMO ELECTRIC INDUSTRIES) 24 September 1986 (1986-09-24)	1-6, 8
A	claims; figures	9, 10
A	✓ EP 0 336 608 A (NEUROMEDICAL SYSTEMS INC) 11 October 1989 (1989-10-11)	
P, A	✓ WO 99 08091 A (ONCOMETRICS IMAGING CORP) 18 February 1999 (1999-02-18)	
A, P	✓ US 5 848 177 A (MACKENZIE CHARLES D ET AL) 8 December 1998 (1998-12-08)	
A	✓ US 5 073 857 A (ELMERICK DONALD V ET AL) 17 December 1991 (1991-12-17)	
A	✓ US 4 965 725 A (RUTENBERG MARK R) 23 October 1990 (1990-10-23)	
	--- -/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

19 October 1999

Date of mailing of the international search report

25/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Coucke, A

INTERNATIONAL SEARCH REPORT

International Application No

T/DK 99/00458

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>✓ PATENT ABSTRACTS OF JAPAN vol. 018, no. 387 (P-1773), 20 July 1994 (1994-07-20) & JP 06 109979 A (SHIMADZU CORP), 22 April 1994 (1994-04-22) abstract</p> <p>---</p>	1-10
Y	<p>✓ PATENT ABSTRACTS OF JAPAN vol. 012, no. 133 (P-693), 22 April 1988 (1988-04-22) & JP 62 254034 A (YOJI UMETANI; OTHERS: 01), 5 November 1987 (1987-11-05) abstract</p> <p>---</p>	1-10
A	<p>✓ PATENT ABSTRACTS OF JAPAN vol. 1998, no. TEX, 31 March 1998 (1998-03-31) & JP 09 322756 A (MORITEX CORP; RES DEV CORP OF JAPAN), 16 December 1997 (1997-12-16) abstract</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PT/DK 99/00458

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0195088	A	24-09-1986	JP 1453904 C	10-08-1988
			JP 61074570 A	16-04-1986
			JP 62060061 B	14-12-1987
			JP 1453905 C	10-08-1988
			JP 61073065 A	15-04-1986
			JP 62060071 B	14-12-1987
			JP 1453906 C	10-08-1988
			JP 61074571 A	16-04-1986
			JP 62060062 B	14-12-1987
			JP 1453907 C	10-08-1988
			JP 61074572 A	16-04-1986
			JP 62060072 B	14-12-1987
			DE 3586892 A	21-01-1993
			WO 8601824 A	27-03-1986
			US 5106584 A	21-04-1992
<hr/>				
EP 0336608	A	11-10-1989	US 4965725 A	23-10-1990
			AT 140327 T	15-07-1996
			AU 628342 B	17-09-1992
			AU 3541589 A	03-11-1989
			BG 51463 A	14-05-1993
			CA 1323700 A	26-10-1993
			CN 1037035 A,B	08-11-1989
			DE 68926796 D	14-08-1996
			DE 68926796 T	07-11-1996
			DK 262490 A	01-11-1990
			ES 2090033 T	16-10-1996
			FI 101653 B	31-07-1998
			GR 3021252 T	31-01-1997
			HK 1003583 A	30-10-1998
			JP 4501325 T	05-03-1992
			RO 106931 A	30-07-1993
			SG 46454 A	20-02-1998
			RU 2096827 C	20-11-1997
			WO 8909969 A	19-10-1989
			US 5544650 A	13-08-1996
			US 5287272 A	15-02-1994
			US 5740270 A	14-04-1998
			US 5939278 A	17-08-1999
<hr/>				
WO 9908091	A	18-02-1999	AU 8724598 A	01-03-1999
<hr/>				
US 5848177	A	08-12-1998	NONE	
<hr/>				
US 5073857	A	17-12-1991	US 5077806 A	31-12-1991
			CA 2014979 A	01-12-1990
<hr/>				
US 4965725	A	23-10-1990	AT 140327 T	15-07-1996
			AU 628342 B	17-09-1992
			AU 3541589 A	03-11-1989
			BG 51463 A	14-05-1993
			CA 1323700 A	26-10-1993
			CN 1037035 A,B	08-11-1989
			DE 68926796 D	14-08-1996
			DE 68926796 T	07-11-1996
			DK 262490 A	01-11-1990
			EP 0336608 A	11-10-1989
			ES 2090033 T	16-10-1996

WO 00/13609 A3

INTERNATIONAL SEARCH REPORT

International Application No

PL /DK 99/00458

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12M1/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A A P,A A,P A A	EP 0 195 088 A (SUMITOMO ELECTRIC INDUSTRIES) 24 September 1986 (1986-09-24) claims; figures --- EP 0 336 608 A (NEUROMEDICAL SYSTEMS INC) 11 October 1989 (1989-10-11) --- WO 99 08091 A (ONCOMETRICS IMAGING CORP) 18 February 1999 (1999-02-18) --- US 5 848 177 A (MACKENZIE CHARLES D ET AL) 8 December 1998 (1998-12-08) --- US 5 073 857 A (ELMERICK DONALD V ET AL) 17 December 1991 (1991-12-17) --- US 4 965 725 A (RUTENBERG MARK R) 23 October 1990 (1990-10-23) --- -/-	1-6,8 9,10

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

19 October 1999

Date of mailing of the international search report

25/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Coucke, A

INTERNATIONAL SEARCH REPORT

International Application No

PC./DK 99/00458

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 018, no. 387 (P-1773), 20 July 1994 (1994-07-20) & JP 06 109979 A (SHIMADZU CORP), 22 April 1994 (1994-04-22) abstract ---	1-10
Y	PATENT ABSTRACTS OF JAPAN vol. 012, no. 133 (P-693), 22 April 1988 (1988-04-22) & JP 62 254034 A (YOJI UMETANI; OTHERS: 01), 5 November 1987 (1987-11-05) abstract ---	1-10
A	PATENT ABSTRACTS OF JAPAN vol. 1998, no. TEX, 31 March 1998 (1998-03-31) & JP 09 322756 A (MORITEX CORP; RES DEV CORP OF JAPAN), 16 December 1997 (1997-12-16) abstract -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 99/00458

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0195088	A	24-09-1986	JP 1453904 C	10-08-1988
			JP 61074570 A	16-04-1986
			JP 62060061 B	14-12-1987
			JP 1453905 C	10-08-1988
			JP 61073065 A	15-04-1986
			JP 62060071 B	14-12-1987
			JP 1453906 C	10-08-1988
			JP 61074571 A	16-04-1986
			JP 62060062 B	14-12-1987
			JP 1453907 C	10-08-1988
			JP 61074572 A	16-04-1986
			JP 62060072 B	14-12-1987
			DE 3586892 A	21-01-1993
			WO 8601824 A	27-03-1986
			US 5106584 A	21-04-1992
EP 0336608	A	11-10-1989	US 4965725 A	23-10-1990
			AT 140327 T	15-07-1996
			AU 628342 B	17-09-1992
			AU 3541589 A	03-11-1989
			BG 51463 A	14-05-1993
			CA 1323700 A	26-10-1993
			CN 1037035 A, B	08-11-1989
			DE 68926796 D	14-08-1996
			DE 68926796 T	07-11-1996
			DK 262490 A	01-11-1990
			ES 2090033 T	16-10-1996
			FI 101653 B	31-07-1998
			GR 3021252 T	31-01-1997
			HK 1003583 A	30-10-1998
			JP 4501325 T	05-03-1992
			RO 106931 A	30-07-1993
			SG 46454 A	20-02-1998
			RU 2096827 C	20-11-1997
			WO 8909969 A	19-10-1989
			US 5544650 A	13-08-1996
			US 5287272 A	15-02-1994
			US 5740270 A	14-04-1998
			US 5939278 A	17-08-1999
WO 9908091	A	18-02-1999	AU 8724598 A	01-03-1999
US 5848177	A	08-12-1998	NONE	
US 5073857	A	17-12-1991	US 5077806 A	31-12-1991
			CA 2014979 A	01-12-1990
US 4965725	A	23-10-1990	AT 140327 T	15-07-1996
			AU 628342 B	17-09-1992
			AU 3541589 A	03-11-1989
			BG 51463 A	14-05-1993
			CA 1323700 A	26-10-1993
			CN 1037035 A, B	08-11-1989
			DE 68926796 D	14-08-1996
			DE 68926796 T	07-11-1996
			DK 262490 A	01-11-1990
			EP 0336608 A	11-10-1989
			ES 2090033 T	16-10-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC 1/DK 99/00458

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4965725 A		FI 101653 B	31-07-1998
		GR 3021252 T	31-01-1997
		HK 1003583 A	30-10-1998
		JP 4501325 T	05-03-1992
		RO 106931 A	30-07-1993
		SG 46454 A	20-02-1998
		RU 2096827 C	20-11-1997
		WO 8909969 A	19-10-1989
		US 5544650 A	13-08-1996
		US 5287272 A	15-02-1994
		US 5740270 A	14-04-1998
		US 5939278 A	17-08-1999
JP 06109979 A	22-04-1994	NONE	
JP 62254034 A	05-11-1987	JP 1926103 C	25-04-1995
		JP 6052227 B	06-07-1994
JP 09322756 A	16-12-1997	US 5952651 A	14-09-1999

Not entered
(Missing claim)

PATENT COOPERATION TREATY

PCT

15
RECD 10 OCT 2000

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P9757PC00	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/DK99/00458	International filing date (day/month/year) 01/09/1999	Priority date (day/month/year) 02/09/1998
International Patent Classification (IPC) or national classification and IPC A61F2/02		
Applicant LANGERHANS ApS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 6 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 12 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 09/03/2000	Date of completion of this report 06.10.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Dietz Schlereth, D Telephone No. +49 89 2399 7488 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK99/00458

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-3,5-12,14,16-22, 25-27 as originally filed

4,4a,13,15,23, 24 as received on 05/08/2000 with letter of 03/08/2000

Claims, No.:

7 as originally filed

1-5,6,8 as received on 05/08/2000 with letter of 03/08/2000

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

* Claim 6 partially as filed with letter of 03.08.00 (page 30)
and partially as originally filed (page 31).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/DK99/00458

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

item V.

1.) Reference is made to the following documents:

D1: EP-A-0 195 088

D2: JP-A-06 109 979 (Patent Abstracts of Japan, vol. 018, No 387, P-1773)

D3: JP-A-62 254 034 (Patent Abstracts of Japan, vol. 012, No 133, P-693)

2.) D1, which is considered to be the closest state of the art discloses an apparatus for sorting cells which comprises a pipette, a manipulator that holds said pipette, a fractional sampling and pouring part, and a controller (see Abstract; p. 1, l. 1-10; p. 6, l. 18-26; p. 7, l. 1-12; p. 9, l. 6-14; p. 12, l. 12-26; p. 13, l. 1-6, 19-26; p. 16, l. 8-19; p. 17, l. 3-14; p. 20, l. 21-26; p. 21, l. 1-26; p. 22, l. 1-23; Embodiments 3 and 4; Figs. 9-10).

The subject-matter of claim 1 differs therefrom in that the apparatus comprises a capillary tube device which picks out specific particles from a first container and
★ transports them to a second container.

In the light of the closest prior art, the technical problem to be solved by the present application was to provide an alternative apparatus for isolating cells.

The solution proposed in claim 1 (and 2-7 as dependent thereon) can be considered novel and inventive within the sense of Art. 33 (2) and (3) PCT for the following reasons:

In the apparatus of claim 1 particles to be selected are detected by a camera system (digital imaging processing) and picked out (and transferred) from a solution by means of a tube device which has capillary dimensions larger than the size of the particles of interest. The apparatus of claim 1 enables specific particles (i) to be detected directly without the need of any other chemical or physical treatment which may damage the particles, (ii) to be isolated with a high efficiency and purity, and (iii) to be transferred automatically in a fast and reliable way. Because of all these characteristics the apparatus of claim 1 is particularly suited for isolating cell clusters from a suspension of cell tissues, such as Islets of Langerhans (see description, pages 6-7).

Transferring a predetermined number of individual cells with the apparatus of D1 requires: (i) counting the number of cells in the solution by using a microscope connected to a TV camera or by measuring the resistance of the solution, and (ii) diluting the solution accordingly in order to adjust the volume of the pipette tip to the desired amount of cells to be transferred (see pages 25-26). It can be inferred from the disclosure of this document, that the injecting/sucking module of the apparatus of D1 comprises pipette tips of common dimensions, that means pipettes which do not have a portion of capillary dimensions.

D2 discloses a micromanipulator system in which a substance can be injected into an individual cell by means of a first micropipette while holding said cell at a fixed position on the tip surface of a second micropipette by suction. The position the micropipettes is controlled by recording and processing images taken with a TV camera (see Abstract and Figure).

D3 discloses an apparatus for measuring with high accuracy very small injection amounts of a substance. The volume of substance comprised in the micropipette is calculated by reading the moving quantity of the meniscus and the diameter dimension of the meniscus position by image processing of a microscopic image (Abstract).

The dimensions of the inner diameter of the micropipettes of D2 cannot be larger than the size of the cell to be treated, otherwise the cell would be sucked inside the suction pipette and would not be accessible for the injection pipette. The device of D3 is thought for measuring small volumes of a liquid sample and therefore any dimension of the inner diameter of the pipette which leads to a measurable meniscus could be used.

Therefore, the skilled person confronted with the above mentioned technical problem and equipped with the teaching of D1-D3 would have had neither motivation nor technical guidance for modifying the apparatus of D1 by implementing a capillary tube device for picking out/transferring particles and arrive at an apparatus according to claim 1.

3.) The subject-matter of independent claim 8 relates to the use of the apparatus of claims 1-7 and is considered to be novel and inventive within the sense of Art. 33 (2) and (3) PCT for analogous reasons as discussed in item 2. above.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/DK99/00458

item VI.

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
US-A-5,848,177	08.12.98	27.02.97	----
WO-A-99/08091	18.02.99	06.08.98	08.08.97*

* priority not checked

The above mentioned documents could be relevant in the case that the claimed priority date of 02.09.98 could not be acknowledged.

25 JULI 2000

LIST OF NUMBERS

1. camera
2. computer
- 5 3. first container, petri dish
4. pipette
5. piston moving electrical motor or piezo unit
6. piston
7. pipette mantle
- 10 8. transport stage
9. transport tube
10. second container, culture flask
11. suction device
12. capillary end section, pipette tip
- 15 13. volume around pipette tip
14. transport tube
15. pipette system
16. core, pipette model 4
17. hole inlet, pipette model 4
- 20 18. hole for outlet pipette model 4
19. hole for tip pipette model 4
20. reservoir
21. transparent section of first container
22. first transport section of first container, silicone tubing
- 25 24. micro pump or valve
25. side tubing
26. second transport section of first container, silicone tubing
27. waste container
- 30 28. further tube
29. mantle of pipette model 4
30. outlet

25 JULI 2000

- 31.inside of mantle
- 32.inlet
- 33.centre of pipette model 4
- 34.outlet side tubing
- 5 35.inlet side tubing
- 36.channel in piston
- 37.inclined front end of piston
- 38.angle between front end of piston and direction of piston movement
- 39.pipette chamber
- 10 40.channel in core of pipette model 4
- 41.turning device
- 42.turning axle
- 43.mantle of reservoir
- 44.first membrane
- 15 45.first volume
- 46.upper wall of reservoir
- 47.rim of first membrane
- 48.inner wall of mantle of reservoir
- 49.second membrane
- 20 50.second volume
- 51.third volume
- 52.upper wall of reservoir
- 53.moving device
- 54.outlet
- 25 55.pump
- 56.inlet
- 57.feeding tube

25 JULI 2000

Figure 6 shows pipette model 4, which is a flow-through pipette with continuous transfer. In this embodiment of the invention, the pipette (4) consists of a cylindrical mantle (29) containing a cylindrical core (16) configured such as to function as a three-way valve. A projection of it is shown in figure 6c. The core (16) can be turned
5 stepwise inside the mantle (29) by a turning device (41), for example an electrical motor or a piezo-unit, which is connected to the core (16) via a turning axle (42).

This pipette (4), as well, ensures fast pipetting and flow-through for transfer of the cell clusters from the pipette tip (12) to the transport tube (14). The core (16) and the
10 mantle (29) can be made of any suitable material, e.g. metal or plastic. For the purpose of supervising the process, it is preferable that the material is transparent. The mantle (29) is constructed with three holes (17, 18, 19) pointing towards the centre (33) of the pipette core (16). The holes (17, 18, 19) are positioned with a mutual angular distance of 120° .

15

The core (16) is constructed with an internal channel (40). The channel (40) has approximately the same diameter as the diameter of the holes (17, 18, 19). The core (16) can be positioned such that it connects two of the three holes, for example the inlet (17) and the outlet (18). In this position, there is a flow of culture medium from a
20 medium supply connected to the inlet (32) through the core channel (40) to the transport tube (14).

In the second position, as shown in figure 6b, which is a 120° turn of the core (16), a connection between the pipette tip (12) and the transport tube (14) is established to
25 suck islets through the internal core channel (40) into the transport tube (14).

The turning of the core is fast, so that only a small volume of medium is sucked into the tip (12) and the channel (40) together with the islet.

30 The apparatus I may be equipped with any type of pipette suitable for sucking in islets. Thus, pipettes based on the well known Pelletier-principle can be mounted as well. A pipette of this type allows for a transfer of a limited number of clusters per

tip (12) in e.g. plastic or by punching out plastic. If a transparent material is used, this may also be used for sending light through the pipette, which can help positioning the pipette tip (12) in the right position. A piston (6) inside the pipette (4) can also be made of any material, but Teflon[®] is preferred due to low friction between the inside
5 (31) of the pipette (4) and the piston (6). For movement of the piston (6) inside the pipette (4), the piston (6) is attached to an electromagnet or piezo-element (5).

The pipette (4) allows suction of the islet through the tip (12) which has an inner diameter that is a little larger than the largest cell clusters to be isolated (specifically, for
10 most islet preparations approximately 250-350 micrometer is optimal). The tip (12) of the pipette (4) can be any length, but to ensure that only a small volume of suspension is sucked into the pipette each time cell clusters are isolated, a length of approximately 10 mm is preferred.

15 Islets are sucked into the pipette when the piston is moved from a first position, where the piston is moved as far as possible into the pipette mantle, outwards to a second position. By performing the outward movement of the piston in small steps, many islets can be sucked into the pipette, before the content inside the pipette is transferred to the second container, for example a culture flask.

20 Alternatively, only one islet is sucked into the pipette and transported to the second container. This method is slower but avoids clogging inside the pipette.

Figure 4 shows pipette model 2, which is a flow-through pipette without continuous
25 transfer. In this embodiment of the invention, the pipette (4) is equipped with a hole for outlet (30) and a short side tube (34) for connection of a transport tube (14) to the pipette (4). The front end (37) of the piston (6) is cut-off with an angle (38) with respect to the moving direction of the piston (6), for example 45°. For movement of the piston (6) inside the pipette (4), the piston (6) is attached to an electromagnet or piezo-
30 element (5).

25 JULI 2000

It is obvious that within the area of animal islet research none of these methods are widely used and the manual islet isolation is still very common and used by most laboratories including those in the pharmaceutical industry. It is clear, therefore, that efforts should be put into facilitation of isolation process since substantial resources are used on this process. In addition, advantages are increased quality of the isolated products and the invention will also reduce inter-operator variation in selection of islets. Improved methods for isolation of rodent islets are useful for the research regarding transplantation and improved methods in this are likely to rub off on the human islet transplantation.

Other systems for sorting cells are known, for example from European patent application EP 195 088, from Japanese patent application JP 06 109 979, and from Japanese patent application JP 62 254 034.

It is an object of the present invention to substantially improve the isolation process. It takes advantage of, and incorporates improvements, of the conventional methods for disintegration of the tissue of interest. It may be used for primary isolation of islets from the surrounding tissue but may also be used for later purification or transfer of islets from one place to another. The primary goal is to provide a fast, reliable apparatus for isolation of pancreatic islets of Langerhans and in the same apparatus implement documentation.

SUMMARY OF THE INVENTION.

The invention is concerned with isolation of cell clusters embedded in a tissue suspension. The apparatus is preferably designed for isolation of pancreatic islets but is applicable to many kinds of cell clusters, single cells, for example spermatozoa, or non biological particles. It takes advantage of conventional methods for disintegration of the primary tissue containing the cell clusters of interest. The principle of the invention is that the apparatus automatically detects the cell clusters in the tissue suspension and subsequently isolate and transfers them to another location. The detection of the

25 JUL 2000

4a

cell clusters is carried out by digital image processing followed by isolation by means of either a moving pipette or performed in a capillary tubing.

BRIEF DESCRIPTION OF THE DRAWINGS.

- 5 Figure 1. Apparatus I in schematic form.

CLAIMS

1. Apparatus for automatic isolation of particles, comprising:

- a reservoir containing a solution with particles;
- 5 - a first container into which an amount of said solution with said particles is fed from said reservoir;
- a second container for accumulation of specific ones of said particles;
- a capillary tube device for picking out one of said specific particles from said first container and for transporting said specific particles to said second container, wherein
- 10 said capillary tube device comprises a transparent section with an inner cross section larger than said specific particles ;
- a computer system;
- a camera system for recording images of said particles and transferring those images digitally to said computer system, where the field of view of the camera contains said
- 15 transparent section;
- a computer program implemented in said computer system, which computer program evaluates said images, identifies and selects said specific particle from said images by predetermined parameters, protocols physical/chemical and/or biochemical characteristics of said specific particle, and controls said picking out and said transport of said
- 20 specific particle.

2. Apparatus according to claim 1, wherein

- said first container comprises a tube with a transparent section, said tube being connected to said reservoir;
- 25 - said tube has an internal flow of said solution with particles from said reservoir;
- said camera system records images of said particles in said transparent section;
- said capillary tube device comprises a micro pump, which micro pump is connected to said tube, and which micro pump by signal from said computer system picks out said specific particle from said solution by pumping a small portion of said solution
- 30 containing said specific particle into a side-tube, which side-tube is connected to the second container.

05-08-2000

DK 009900458

REPLACEMENT SHEET

28a

25 JULI 2000

3. Apparatus according to claim 1, wherein said capillary tube device comprises:

AMENDED SHEET

- a pipette with a transparent capillary end section for picking out said specific particle from said first container, said pipette comprising a piston inside said pipette that is moved by a moving device, which moving device is controlled by said computer system with said computer program, wherein the volume of the pipette chamber is changed by the movement of said piston;

- a transport stage with motors for moving said pipette to the position of said specific particle and, after picking up said specific particle, transporting said specific particle to said second container, which transport stage is controlled by said computer with said computer program.

4. Apparatus according to claim 1, wherein said capillary tube device comprises:

- a pipette with a transparent capillary end section for picking out said specific particle from said first container by sucking out of said first container a small portion of solution that contains said particle;

- a transport tube connected to said pipette for transporting said specific particle from said capillary end section to said second container;

- a piston inside said pipette, wherein the volume of the pipette chamber is changed by the movement of said piston;

- a moving device to move said piston between a first and a second position, which moving device is controlled by said computer system with said computer program determining the first and second position, where said piston in first position inhibits and in the second position establishes a connection between the capillary end section and the transport tube;

- a suction device creating a lower pressure in said second container and in said transport tube, such that, when said piston is in said second position, solution is transported from said capillary end section into said transport tube;

- a transport stage with motors for moving said pipette with said capillary end section to position of said selected specific particle in said solution in said first container, which transport stage is controlled by said computer with said computer program.

5. Apparatus according to claim 1, wherein said capillary tube device comprises

29a

25 JULI 2000

- a pipette with a transparent capillary end section for picking out said specific particle from said first container by sucking out of said first container a small portion of solution that contains said particle;

- a transport tube connected to said pipette for transporting said specific particle from said capillary end section to said second container;

- a supplier tube connected to said pipette;

5 - a piston in side said pipette, wherein the volume of the pipette chamber is changed by the movement of said piston;

- a moving device to move said piston between a first and a second position, which moving device is controlled by said computer system with said computer program determining said first and said second position, where said piston in said second position establishes a connection between the capillary end section and the transport tube and inhibits the connection between the supplier tube and the transport tube;

10 - a hollow channel in said piston, which channel, when said piston is in said first position, inhibits the connection between said capillary end section and any of these two said tubes but connects said supplier tube with said transport tube such that fluid can flow from said supplier tube to said transport tube;

15 - a suction device creating a lower pressure in said second container and in said transport tube, such that, when said piston is in said first position, solution is transported from said supplier tube to said transport tube, and when said piston is in said second position, solution is transported from said capillary end section into said transport tube;

20 - a transport stage with motors for moving said pipette with said capillary end section to position of said selected specific particle in said solution in said first container, which transport stage is controlled by said computer with said computer program.

6. Apparatus according to claim 1, wherein said capillary tube device comprises:

25 - a three-way valve with a turnable cylindrical core connected to
a transparent capillary end section for picking out said specific particle from said first container by sucking out of said first container a small portion of solution that contains said particle,
a transport tube for transporting said specific particle from said capillary end
30 section to said second container; and
a supplier tube;

25 JULI 2000

8. Use of an apparatus according to claim 1-7 for isolation of at least one from the group consisting of specific cell types, cell clusters obtained by treating organic tissue with dissociating methods, and Islets of Langerhans.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

T/DK 99/00458

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4965725 A		FI 101653 B GR 3021252 T HK 1003583 A JP 4501325 T RO 106931 A SG 46454 A RU 2096827 C WO 8909969 A US 5544650 A US 5287272 A US 5740270 A US 5939278 A	31-07-1998 31-01-1997 30-10-1998 05-03-1992 30-07-1993 20-02-1998 20-11-1997 19-10-1989 13-08-1996 15-02-1994 14-04-1998 17-08-1999
JP 06109979 A	22-04-1994	NONE	
JP 62254034 A	05-11-1987	JP 1926103 C JP 6052227 B	25-04-1995 06-07-1994
JP 09322756 A	16-12-1997	US 5952651 A	14-09-1999